

AMENDMENTS TO THE SPECIFICATION

Please amend the specification as shown:

Please delete paragraph [0069] and replace it with the following paragraph:

[0069] Figure 9 Enhanced efficacy of α DEC-205:OVA plus α CD40 relative to other immunization approaches. (A) C57BL/6 mice were immunized s.c. with several methods: spleen DC pulsed *ex vivo* with 10 μ g/mL each of α DEC-205:OVA and α CD40; 500 μ g OVA in CFA; 50 μ g OVA with 25 μ g α CD40; 50 μ g of SIINFEKL peptide (**SEQ ID NO: 15**) with 25 μ g α CD40; or 50 ng of OVA in α DEC-205:OVA with 25 μ g of α CD40. 7 or 30 days later, lymph nodes were harvested and T cell expansion evaluated by K^b-SIINFEKL:PE (**peptide disclosed as SEQ ID NO: 15**) tetramer and CD62L staining. The gate for the y-axis was placed relative to the CD62L negative tetramer binding cells in the right panel. Indicated percentages are percent of CD8⁺ lymphocytes. (B) As in A, but IFN- γ secretion evaluated by intracellular cytokine staining. Data are means of 3 experiments.

Please delete paragraph [0082] and replace it with the following paragraph:

[0082] As used herein, the term "single-chain antibody" refers to a polypeptide comprising a V_H region and a V_L region in polypeptide linkage, generally linked via a spacer peptide (e.g., [Gly-Gly-Gly-Gly-Ser]_x (**SEQ ID NO: 38**)), and which may comprise additional amino acid sequences at the amino- and/or carboxy- termini. For example, a single-chain antibody may comprise a tether segment for linking to the encoding polynucleotide. As an example, a scFv (single chain fragment variable) is a single-chain antibody. Single-chain antibodies are generally proteins consisting of one or more polypeptide segments of at least 10 contiguous amino acids substantially encoded by genes of the immunoglobulin superfamily (e.g., see The Immunoglobulin Gene Superfamily, A. F. Williams and A. N. Barclay, in Immunoglobulin Genes, T. Honjo, F. W. Alt, and T. H. Rabbitts, eds., (1989) Academic Press: San Diego, Calif., pp.361-387, which is incorporated herein by reference), most frequently encoded by a rodent, non-human primate, avian, porcine, bovine, ovine, goat, or human heavy chain or light chain gene sequence. A functional single-chain antibody generally contains a sufficient portion of an

immunoglobulin superfamily gene product so as to retain the property of binding to a specific target molecule, typically a receptor or antigen (epitope).

Please delete paragraph [0164] and replace it with the following paragraph:

[0164] Beginning in 1988, single-chain analogues of Fv fragments and their fusion proteins have been reliably generated by antibody engineering methods. The first step generally involves obtaining the genes encoding V_H and V_L regions with desired binding properties; these V genes may be isolated from a specific hybridoma cell line, selected from a combinatorial V-gene library, or made by V gene synthesis. The single-chain Fv is formed by connecting the component V genes with an oligonucleotide that encodes an appropriately designed linker peptide, such as (Gly-Gly-Gly-Gly-Ser)₃ (**SEQ ID NO: 39**) or equivalent linker peptide(s). The linker bridges the C-terminus of the first V region and N-terminus of the second, ordered as either V_H-linker-V_L or V_L-linker-V_H. In principle, the scFv binding site can faithfully replicate both the affinity and specificity of its parent antibody combining site.

Please delete paragraph [0176] and replace it with the following paragraph:

[0176] The immunoglobulin library is expressed by a population of display packages, preferably derived from filamentous phage, to form an antibody display library. In addition to commercially available kits for generating phage display libraries (e.g., the Pharmacia Recombinant Phage Antibody System, Catalog No. 27-9400-01; and the Stratagene SurfZAP.TM. Phage Display Kit, Catalog No. 240612), examples of methods and reagents particularly amenable for use in generating antibody display library can be found in, for example, Ladner et al. U.S. Pat. No. 5,223,409; Kang et al. International Publication No. WO 92/18619; Dower et al. International Publication No. WO 91/17271; Winter et al. International Publication WO 92/20791; Markland et al. International Publication No. WO 92/15679; Breitling et al. International Publication WO 93/01288; McCafferty et al. International Publication No. WO 92/01047; Garrard et al. International Publication No. WO 92/09690; Ladner et al. International Publication No. WO 90/02809; Fuchs et al. (1991) Bio/Technology 9:1370-1372; Hay et al. (1992) Hum Antibod Hybridomas 3:81-85; Huse et al. (1989) Science 246:1275-1281; Griffiths et al. (1993) EMBO J 12:725-734; Hawkins et al. (1992) J Mol Biol 226:889-896; Clackson et al. (1991) Nature 352:624-628; Gram et al. (1992) Proc. Natl. Acad.

Sci. USA 89:3576-3580; Garrad et al. (1991) Bio/Technology 2:1373-1377; Hoogenboom et al. (1991) Nuc Acid Res 19:4133-4137; and Barbas et al. (1991) Proc. Natl. Acad. Sci. USA 88:7978-7982. As generally described in McCafferty et al. Nature (1990) 348:552-554, complete VH and VL domains of an antibody, joined by a flexible (Gly₄-Ser)₃ (**SEQ ID NO: 39**) linker, can be used to produce a single chain antibody expressed on the surface of a display package, such as a filamentous phage.

Please delete paragraph [0178] and replace it with the following paragraph:

[0178] Once isolated, nucleic acid molecules encoding antibody chains, or portions thereof, can be further manipulated using standard recombinant DNA techniques. For example, a single chain antibody gene can also be created by linking a VL coding region to a VH coding region via a nucleotide sequence encoding a flexible linker (e.g., (Gly₄-Ser)₃ (**SEQ ID NO: 39**)). Single chain antibodies can be engineered in accordance with the teachings of Bird et al. (1988) Science 242:423-426; Huston et al. (1988) Proc. Natl. Acad. Sci USA 85:5879-5883; Ladner, et al. International Publication Number WO 88/06630; and McCafferty, et al. International Publication No. WO 92/10147. A preferred single chain antibody for use in the invention binds to human DEC-205. A plasmid encoding a scFv antibody to DEC-205 would be prepared using standard molecular biological techniques. Another manipulation that can be performed on isolated antibody genes is to link the antibody gene to a nucleotide sequence encoding an amino acid sequence that directs the antibody homologue to a particular intracellular compartment. A preferred nucleotide sequence to which an antibody gene is linked encodes a signal sequence (also referred to as a leader peptide). Signal sequences are art-recognized amino acid sequences that direct a protein containing the signal sequence at its amino-terminal end to the endoplasmic reticulum (ER). Typically, signal sequences comprise a number of hydrophobic amino acid residues. Alternatively, an antibody homologue can be linked to an amino acid sequence that directs the antibody homologue to a different compartment of the cell. For example, a nuclear localization sequence (NLS) can be linked to the antibody homologue to direct the antibody homologue to the cell nucleus. Nuclear localization sequences are art-recognized targeting sequences. Typically, an NLS is composed of a number of basic amino acid residues.

Please delete paragraph [0254] and replace it with the following paragraph:

[0254] Total RNA was prepared from NLDC-145 and GLI7 (gift of R.J. Hodes, National Institutes of Health, Bethesda, MD) hybridomas (both rat IgG2a) using Trizol (GIBCO BRL). Full-length Ig cDNAs were produced with 5'-RACE PCR kit (GIBCO BRL) using primers specific for 3'-ends of rat IgG2a and Ig kappa. The V regions were cloned in frame with mouse Ig kappa constant regions and IgG1 constant regions carrying mutations that interfere with FcR binding (Clynes, R.A., (2000), Nat. Med. 6:443-446). DNA coding for hen egg lysozyme (HEL) peptide 46–61 with spacing residues on both sides was added to the C terminus of the heavy chain using synthetic oligonucleotides. Gene specific primers for cloning of rat IgG2a and Ig kappa: 3'-ATAGTTTAGCGGCCGCGATATCTCACTAACACTCATTCTGTTGAAGCT (SEQ ID NO: 7) ; 3'-ATAGTTTAGCGGCCGCTCACTAGCTAGCTTTACCAGGAGAGTGGGAGAG- ACTCTTCT (SEQ ID NO: 8) ; HEL peptide fragment construction: 5'-CTAGCGACATGGCCAAGAAGGAGACAGTCTGGAGGCTCGAG-GAGTTCGGTAGGTTTCACAAACAGGAAC (SEQ ID NO: 9) ; 5'-acagacgtagcacagactatggtattctccagattaacagcaggtattatgacggtaggacatgataggc (SEQ ID NO: [[10]]40) ; 3'-gctgtaccggttctctctgtcagacctccgagctcctcaa-gccatccaagtgtttgtccttgtgtctg (SEQ ID NO: 11) ; 3'-CCATCGTGTCTGATACCATAAGAGGTCTAATTGTCGTCCATAATACTGCCATCCTGT ACTATCCGCCGG (SEQ ID NO: 12). The anti-DEC-205 V region DNA sequences for the lambda and the heavy chains of the antibody can be found in Figure 13, as SEQ ID NOS: 13 and 14. The anti-human CD40 antibody sequence can be found as SEQ ID NOS: 17, 18 and 19.

Please delete paragraph [0260] and replace it with the following paragraph:

[0260] 5'ATAGTTTAGCGGCCCGCGATATCTCACTAACACTCATTCTGTTGAAGCT (SEQ ID NO:[[7]]41); 3'ATAGTTTAGCGGCCGCTCACTAGCTAGCTTTACCAGGAGAGTGGGAG- AGAC TCTTCT (SEQ ID NO:8).

Please delete paragraph [0288] and replace it with the following paragraph:

[0288] Assays for OVA immunization. Proliferation of primed CD4⁺ or CD8⁺ T cells was evaluated by labeling bulk spleen suspensions with CFSE as above (but at 1 μ M) and restimulating with LPS-free OVA (500 μ g/mL) for 5 days in 24 well dishes at 2.5×10^6 cells/well. Cultures were then washed, stained for CD4 and CD8 and evaluated for proliferation by flow cytometry. ELISPOT assays were performed by restimulating spleen suspensions for 2 days with H-2K^b-restricted peptide (SIINFEKL (**SEQ ID NO: 15**); 1.0 μ M) or an I-A^b-restricted peptide (LSQAVHAAHAEINEAGR (**SEQ ID NO: 16**); 1.0 μ M). The *in vivo* response of OVA specific CD8⁺ T cells was evaluated by staining with K^b-SIINFEKL:PE (**peptide disclosed as SEQ ID NO: 15**) tetramers (kindly provided by Dr. E. Pamer, Memorial Sloan Kettering Institute) and CD62L for 1 hr at 4°C. Also IFN- γ producing effector cells were evaluated by culturing 5×10^6 lymph node or spleen cells with SIINFEKL peptide (**SEQ ID NO: 15**) (1.0 μ M) for 6 hrs in the presence of brefeldin A (Sigma; 5 μ g/mL). Cells were then harvested, stained for extracellular CD8 and then stained for cytokines with the BD Intracellular Cytokine Staining Starter Kit. *In vivo* CTL assays were performed as described (Ho, et al., (1994), J. Exp. Med. 179:1539-1549) by injecting 1:1 mixtures of peptide-pulsed and unpulsed syngeneic splenocytes (7×10^6 each) and, 12-18 hrs later, specific lysis quantified as $\{(1 - (\text{ratio unprimed} / \text{ratio primed})) \times 100\}$, with ratio determined as $\{\% \text{CFSE}^{\text{lo}} / \% \text{CFSE}^{\text{hi}}\}$ (Wong, P., and E.G. Pamer. (2003), *Immunity* 18:499-511).